# Controlling Damping-off in Seeds and Seedlings Using *Trichoderma* Seed Coating©

**Maree Debenham and Andrew McLachlan**
The New Zealand Institute for Plant & Food Research Limited, Private Bag 11600, Palmerston North 4474

**Craig McGill**
Institute of Natural Resources (PN433), Massey University, Private Bag 11222, Palmerston North 4474
Email: maree.debenham@plantandfood.co.nz

## INTRODUCTION

*Trichoderma* spp. are beneficial fungi that have long been associated with disease control in many crops. Many *Trichoderma* can establish on plant roots and form a symbiotic relationship with the plant. They produce various compounds such as antibiotics and toxic metabolites, or have evolved various techniques such as coiling and parasitism to control plant disease. In addition, the root-fungal association induces a systemic resistance within the plant that can guard against pathogen attack, enhance root development and growth, increase crop productivity, and enhance nutrient uptake (Harman et al., 2004). *Trichoderma* are now widely used in horticulture, particularly the strains that are rhizosphere competent and can colonise plant roots. With increasing concern about the use of fungicides, resistance issues, and environmental and worker safety associated with their use, the desire to use biocontrol agents could well increase. New strains of *Trichoderma* are being categorised each year and several research groups in New Zealand and worldwide are working on biological control using *Trichoderma*. The research involves incorporating *Trichoderma* into various aspects of the production chain from propagation right through to postharvest (Fig. 1).

For example, recent work by the Bio-Protection Research Centre at Lincoln University has been carried out in an effort to control *Botrytis* in strawberries, *Fusarium* in cucumbers, and *Sclerotinia* in onion (McLean et al., 2005; Stewart et al., 2007; Card et al., 2009). In addition, as detailed here, research at Plant & Food Research and Massey University has recently focused on control of damping-off in seeds and seedlings (Debenham, 2010). Several research groups overseas are assessing various strains of *Trichoderma* for commercialisation, combining strains using protoplast fusion, and continuing work on different application methods for using *Trichoderma* at various stages of plant growth (Elad et al., 1999; Harman et al., 2004; Mastouri et al., 2010).

Strains of *Trichoderma* that confer some resistance against the damping-off pathogens of *Pythium* sp. and *Rhizoctonia* sp., have been identified and trialled on seeds and seedlings. Damping-off infection can occur rapidly, often within the first 24 h of planting (Lifshitz et al., 1986; Mckellar and Nelson, 2003). Therefore, it is desirable to have the biocontrol agent located on or near the seed, such as incorporated into a seed coating, to ensure rapid control of any germinating pathogens. However, specific details regarding seed coatings incorporating *Trich-*

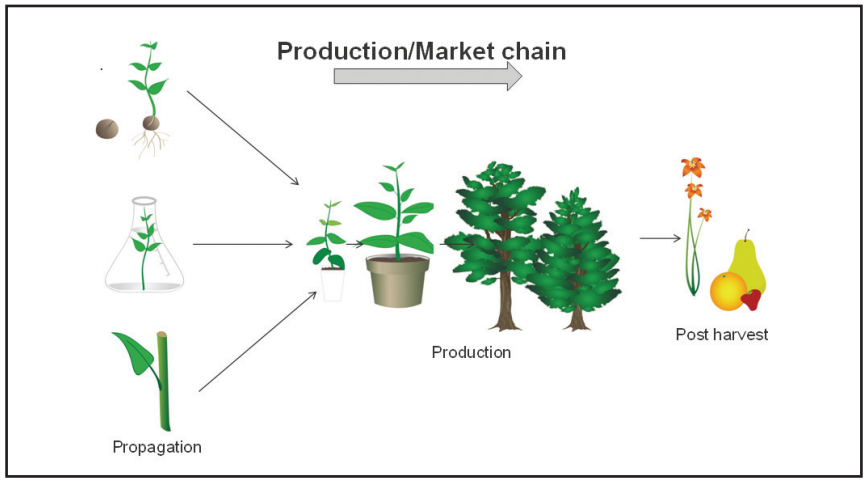


**Figure 1.** Production/market chain indicating areas of current research using *Trichoderma.*

*oderma* are commercially sensitive and not available publicly. Our literature and patent searches identified common components used in seed coating, such as adhesives like Methocel®, gum arabic, and xanthan gum, and fillers such as peat, lime, talc, vermiculite, gypsum, and sphagnum moss (Elad et al., 1982; Harman and Taylor, 1988; Spiegel and Chet, 1998; Howell, 2007). There is a wide range of application rates for *Trichoderma,* but the consensus is between $10^6$ and $10^8$ colony forming units (cfu) per seed (Ahmad and Baker, 1985; Lifshitz et al., 1986; Bennett and Whipps, 2008).

In addition, coating with biocontrol agents poses challenges that agrichemical coating does not have. For instance:

1) Sufficient biocontrol agent must survive the coating process and remain viable to colonise the roots and rhizosphere of the plants.
2) The biocontrol agent must survive storage on the seed.
3) The biocontrol agent may need to be applied in conjunction with insecticides or other fungicides.
4) The biocontrol agent cannot be detrimental to seed storage, germination, or vigour.
5) The coating must adhere well to the seed and not fall off excessively during storage, transit, and handling.
6) *Trichoderma* spores should be applied to the seed at a minimum rate of $10^6$ (cfu) per seed.
7) The initial formulation of the biocontrol agent, varying environmental conditions, pathogen loading, and varying carbon soures on each seed, can all influence the proliferation of *Trichoderma* in the soil surrounding the seed.

Hence this study develops a method of coating several taxa of damping-off-prone seed with *Trichoderma*, then assesses the effectiveness of the coating to control damping-off pathogens.

## MATERIALS AND METHODS

Seven seed lines were used for the trials: *Viola* × *wittrockiana* (pansy), *Lobularia maritima* (alyssum), *Impatiens walleriana*, *Antirrhinum majus, Latuca sativa* (lettuce*), Rudbeckia hirta, Zinnia elegans*; all from Egmont Seeds Ltd, New Plymouth, New Zealand. Before commencing the experiment, the presence of nonpathogenic fungi was confirmed using the method of Debenham (2010).

**Seed Coating.** A coating using Methocel oil, water, gypsum, and *Trichoderma harzianum* Rifai (Agrimm Technologies, Christchurch, New Zealand) spore biomass containing $2 \times 10^{10}$ cfu/g was used. It was made as follows:

1) Mix 2.5 g of Methocel with 2 ml oil in a beaker for 1 min until soft, then add 20 ml water and leave to stand for 5 min until a a gel-like consistency is formed.
2) Remove approximately 2 ml of gel and add to one gram of seed. Stir with spatula to obtain a thin, even coating on each seed.
3) Add *Trichoderma* spore powder to give a final coating of $5 \times 10^6$ cfu/seed and stir until a thin coating is visible on the seed and no spore powder is left in the beaker.
4) Add gypsum, approximately 1 g at a time, and stir until the remainder of the gel is absorbed and the seed are well separated.
5) Tip seed onto a plate and leave to dry at room temperature for 2–4 h.
6) To confirm that the *Trichoderma* spores survived the seed coating process, a *Trichoderma* selective medium was used on the washed coating material from five zinnia seed using the method of Debenham (2010).

**Determining Maximum Germination Potential.** To determine the maximum germination potential of the seed lots, four replicates of 50 coated and uncoated seed of each line were used. Seed were sown on double-layer blue blotters (Steel Blue germination blotters, Anchor Paper Company, St. Paul, Minnesota) and sealed in plastic containers (170 × 120 × 40 mm) before being pre-treated with chilling or $KNO_3$ in accordance with International Seed Testing Association Rules (ISTA, 2011) to alleviate any dormancy requirements. Depending on the species, dormancy breaking involved 5–7 days of moist pre-chilling at 5 °C. After dormancy breaking requirements were met, seed were transferred to a cabinet with a fluctuating temperature of 20–30 °C with 8-h photoperiod during the 30 °C cycle for the required number of days as defined by ISTA (2011). Seed were assessed every 4–7 days for normal seedling development and for any evidence that *Trichoderma* could be causing detrimental effects to the seed or seedlings, such as coiling of the radicle or the shoot, or areas of necrosis. This controlled germination trial in the laboratory provided the optimum conditions; therefore the number of seedlings emerging was considered the maximum germination potential for each seed lot.

**Effectiveness of Seed Coating on Damping-Off Incidence.** Four replicates of 50 seed of each seed species, of both coated and uncoated treatments, were pretreated to alleviate any dormancy as previously described, before being sown in seed-raising trays each containing approximately 1 kg of bark and pumice mix (80 : 20, v/v). This bark : pumice potting mix had been previously screened and was known to contain *Pythium* and *Rhizoctonia.* The trays were placed in a greenhouse

and kept moist by hand-watering and overhead sprinklers. Seed were assessed after 14 days for germination and signs of damping-off disease. Sample seedlings with symptoms of damping-off disease were rinsed in water and placed on agar plates, then incubated at 24±1 °C for 3–7 days before assessment of fungal growth using a dissecting microscope.

**Data Analysis.** The results from each seed line were analysed separately. First, the germination of coated and uncoated seed in laboratory conditions was compared using a binomial generalised linear model with a logit link. From this analysis, separately for the coated and uncoated seed, the mean germination was estimated, along with a measure of variation or uncertainty in this mean. Second, again separately for the coated and uncoated seed from each line, the mean amount of germination lost to disease was calculated simply as the mean laboratory germination minus the mean potting-mix germination. In contrast, the variability in the percent disease was calculated as the variability from the laboratory plus the variability from the potting mix. These two variances were added and then the square root was taken of this sum to get the standard deviation. Finally, once the mean percent disease and variability had been calculated, these values were used in a standard two-sample Student t-test to compare the mean disease rates between the coated and uncoated seed for each seed line.

## RESULTS AND DISCUSSION

There was variable control of damping-off, with zinnia and lettuce showing increased disease incidence with *Trichoderma* seed coating ($P$<0.001; 0.006), alyssum and pansy showing no significant difference ($P$ = 0 45; 0.92), and *Impatiens*, *Antirrhinum*, and *Rudbeckia* showing reduced disease incidence ($P$ = <0.001, 0.001, and 0.007, respectively) (Fig. 2).

In the controlled laboratory environment, seeds of alyssum, pansy, and zinnia showed increased germination percentage when coated with *Trichoderma* (alyssum 80% uncoated vs. 97% coated, $P$ = 0.001), pansy (76% uncoated vs. 86% coated $P$ = 0.010); and zinnia (71% uncoated vs. 81% coated, $P$ = 0.013). Lettuce showed reduced germination (99% uncoated vs. 93%, $P$ = 0.034) (Fig. 3). *Impatiens*, *Rudbeckia*, and *Antirrhinum* showed no difference in germination percentage ($P$ = 1.0, 1.0, 0.89, respectively). There was no evidence of toxicity to any germinating seedlings, and all shoots and radicles were considered normal in appearance.

The seed coating adhered well to the seed and 94% of the spores survived the coating process and remained viable (Fig. 4). Previous trials indicated that spores remained at this degree of viability after 6 months of storage at 5 °C (Debenham, 2010). Fungi isolated from the seed coating were confirmed as *Trichoderma* (Fig. 5).

Successful results using *Trichoderma* to control a certain pathogen in a particular soil, under particular environmental conditions, cannot be extrapolated to imply that it will work just as effectively in a different environment. Unlike fungicides, which often show the same degree of control on a wide spectrum of pathogens, over variable environmental conditions, *Trichoderma* cannot offer that same control. From a commercial grower's point of view, inconsistent control over seasons is difficult to manage and may introduce unnecessary risks to their system. Growers may prefer a product that gives consistent 80% control, to one that sometimes gives 90% control and at other times gives only 50% control (Stewart et al., 2007).

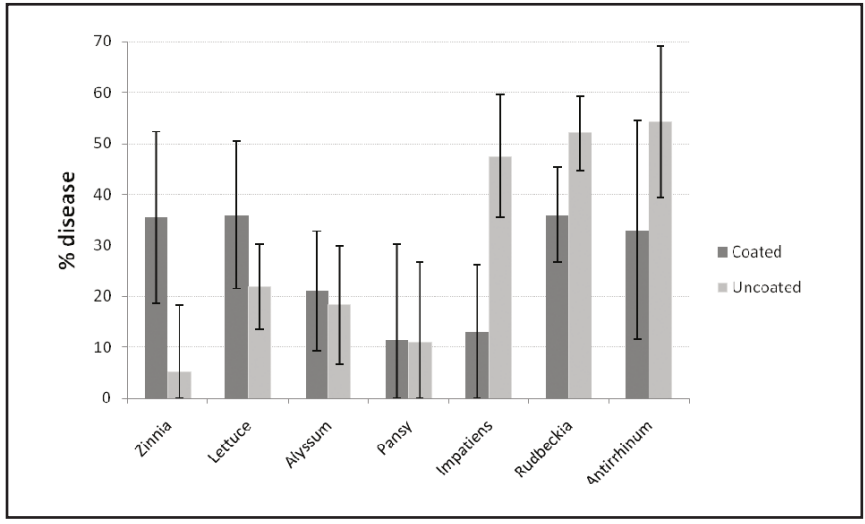


**Figure 2**. Effect of *Trichoderma* seed coating on disease incidence in a range of seedlings. Error bars indicate 95% confidence intervals.

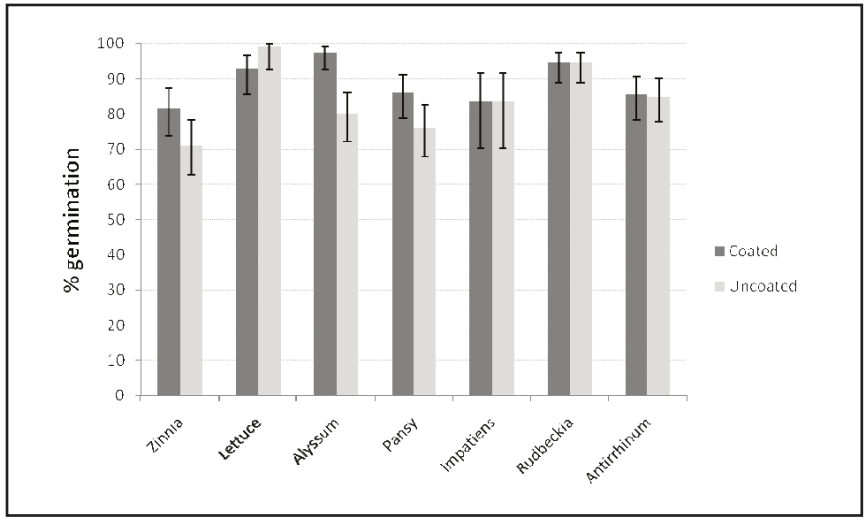


**Figure 3.** Germination of different seed lines whether coated or noncoated with *Trichoderma* (error bars indicate 95% confidence intervals).

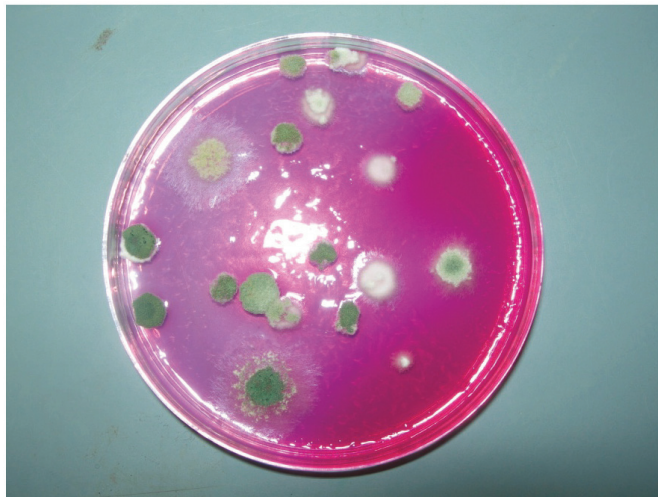

**Figure 4.** Colonies of *Trichoderma* growing on selective medium.

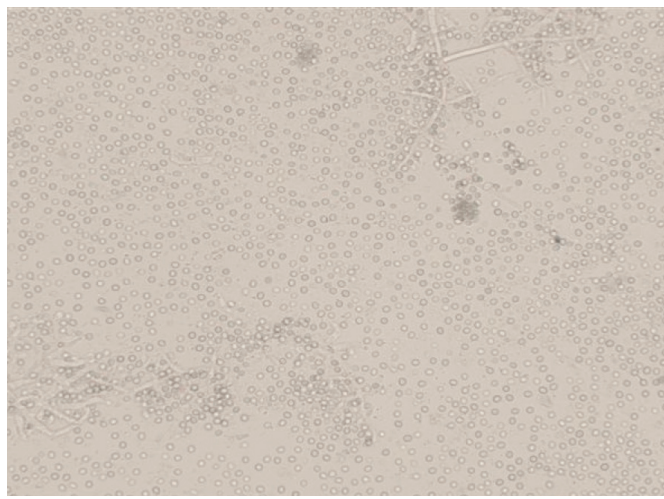

**Figure 5.** *Trichoderma* conidia isolated from selective medium (400 × magnification).

These findings are consistent with other studies that show variable control of disease using *Trichoderma*-based products (Bell et al., 2000; Harman, 2000; Keinath et al., 2000; Stewart et al., 2007) and highlights the limitations of biological control, and that even under closely monitored environmental conditions, *Trichoderma* has performed inconsistently. Under usual growers' conditions, which will have variable temperatures, moisture, light, pathogen loadings, different pathogenic species, and different soils or potting mixes, from those in this trial, it seems likely that *Trichoderma* performance would be inconsistent as well. Inconsistent control is one reason why the use of biocontrol agents, including *Trichoderma,* has not been embraced by industry. An holistic, integrated approach to the use of biocontrol agents may be required, perhaps involving combinations of different strains of biocontrol agents, or biocontrol agents with fungicides, to produce a more encompassing means of disease control.

Other beneficial aspects of *Trichoderma* that have not been quantified in this study are enhanced plant growth and production. Many growers have expressed anecdotal evidence of seeing enhanced growth when they have used *Trichoderma* products in their production systems. Hence, the use of *Trichoderma*, despite not being demonstrated to control damping-off pathogens in seed lines consistently, could still have considerable value in enhanced plant growth and disease resistance.

## CONCLUSION

Seeds were successfully coated with *Trichoderma* spores, using Methocel®, gypsum and oil in a simple coating method. The coating adhered well to the seed, and *Trichoderma* conidial viability remained high. While the results were inconsistent, the potential of *Trichoderma* to control damping-off, plus possible benefits to subsequent plant growth and production, suggest that further research should be undertaken.

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